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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/048577 A1

(54) Title: INHIBITORS OF GST A3-3 AND GST A1-1 FOR THE TREATMENT OF CANCER

(57) Abstract: The present invention relates to a novel drug target, glutathione transferase (GST), preferably GST A3-3, as target for treatment of cancer and other diseases responsive to inhibition of steroid hormone production. The present invention also relates to a method for screening of compounds or drug candidates that modulate, preferably inhibit, the activity of GST, in which method GST is used as a drug target. The invention further relates to the use of inhibitors of GST A3-3 for production of a drug for treatment of steroid hormone dependent diseases, such as for treatment of cancer, preferably prostate cancer or breast cancer. The present invention also relates to a method for treating cancer or steroid hormone dependent diseases, comprising administering, e.g., a non-steroidal compound that modulates the tissue concentration of GST A3-3 or inhibits the enzymatic activity of GST A3-3, to a human in need of such a treatment.

NOVEL DRUG TARGET

Field of the invention

The present invention relates to a novel drug target. More precisely, glutathione transferase (GST) as target for treatment of cancer and other diseases responsive to inhibition of steroid hormone production. Preferably, the GST is GST A3-3 with steroid isomerase activity.

Background of the invention

Prostate cancer and breast cancer are two major forms of malignant disease, which affect a significant proportion of the population. Tumor growth in both cases is often dependent on steroid hormones and an important therapeutic approach involves ablation of hormone production and blockage of the hormone receptor.

Steroid hormone biosynthesis proceeds from cholesterol to androgens (e.g. testosterone and dihydrotestosterone) and estrogens (e.g. progesterone and estradiol) via a series of metabolic intermediates. An obligatory step in each pathway leading to the respective hormones involves the isomerization of the Δ^5-double bond to the Δ^4-double-bond in the steroid structure. The isomerization is preceded by oxidation of the 3β-hydroxy compound into a 3-keto steroid, catalyzed by 3β-hydroxysteroid dehydrogenase. This dehydrogenase has been shown to have an associated steroid isomerase activity.

Glutathione transferases, GSTs, occur in multiple forms (1) and are present in all cellular fractions. The mammalian GSTs can be divided into soluble and membrane-bound enzymes. They are traditionally regarded as detoxication enzymes constituting the main cellular defense against electrophilic compounds that cause mutations, cancer and other degenerative diseases. However, the number of homologous GST genes in eukaryotic cells, including human, has been estimated to exceed 30, and it is becoming clear that some GSTs have other specific roles in relation to physiologically relevant substrates. Therefore, it is misleading to consider GSTs as limited to general detoxication of electrophiles, since some GSTs have roles in the metabolism of well-defined cellular substrates. The recently discovered GST A3-3 appears to have such a different role in double-bond isomerizations of steroids in hormone biosynthesis and should properly be regarded as a steroid isomerase rather than a detoxication enzyme (2).

The enzyme is present in steroidogenic organs such as testis, ovary, placenta and the adrenal gland, but not in significant amounts in other tissues such as liver, thymus, skeletal muscle and brain (2). A putative GST in the human adrenal cell line H295R is markedly induced by adrenocorticotropic hormone (ACTH), a pituitary peptide that stimulates steroid hormone synthesis (3).

It is known that GSTs functioning as cellular detoxication enzymes are inhibited by a wide variety of agents *in vitro* (1). The different GSTs differ widely in their sensitivities to the inhibitors, whereby a given GST may be strongly inhibited by a compound that has no effect on another GST. Some GST inhibitors have been shown to be effective in cellular systems and in clinical trials. However, inhibition data have not previously been obtained for the recently discovered GST A3-3/steroid isomerase (2) and known inhibitors may be ineffective in the steroid isomerase reaction.

Summary of the invention

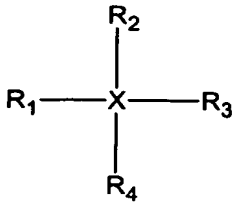
According to the present invention glutathione transferase (GST), preferably GST A3-3/steroid isomerase, is provided as a new target for chemotherapy, based on its contribution to double-bond isomerizations in steroid biosynthesis. GST A3-3 has selective tissue distribution and shows high catalytic activity in the isomerization of both Δ^5-androstene-3,17-dione and Δ^5-pregnene-3,20-dione (Fig. 1). The present inventor has shown that the catalytic efficiency of GST A3-3 is 200-fold higher than the steroid isomerase activity of 3β-hydroxysteroid dehydrogenase. The invention is primarily concerned with cancer in the prostate, but the principle of inhibiting steroid hormone production is also applicable to steroid-responsive cancer in the breast and in other organs. Further, it is applicable to other steroid hormone-dependent diseases such as Cushing's syndrome.

Thus, in a first aspect the invention relates to the use of glutathione transferase (GST) as a drug target for screening of compounds that inhibit the activity of GST for treatment of steroid hormone dependent diseases, such as for treatment of cancer, preferably prostate cancer and breast cancer. Inhibition of activity is also meant to include reduction of the tissue level of catalytically active GST protein by inhibiting its biosynthesis or promoting its degradation.

The GST is preferably GST A3-3. Preferably, pharmaceutically acceptable compounds, which inhibit the activity of GST A3-3 or GST A1-1, are screened for. Thus, the present invention relates to a method for screening of compounds or drug candidates that modulate, preferably inhibit, GST in which method GST is used as a drug target. Such a screening assay may for example be performed as in high throughput screening.

In a second aspect, the invention relates to the use of inhibitors of GST A3-3 or GST A1-1 identifiable by said screening method as a medicament. Said medicament can be used for treatment of steroid hormone dependent diseases, such as for treatment of cancer, preferably the cancer is prostate cancer or breast cancer.

Examples of compounds to be used according to the invention include GST inhibitors having the following formula:



wherein R_1, R_2, R_3 and R_4 can be alkyl groups, such as methyl, ethyl, propyl, butyl, pentyl, hexyl; aryl groups, such as phenyl or substituted phenyl, preferably substituted with lower alkyl, hydroxyl or alkoxy groups; or chemical derivatives or combinations of these groups; the R_1, R_2, R_3 and R_4 groups can be linear; branched, such as substituted with lower alkyl, hydroxyl or alkoxy groups; or cyclic, such as cyclopentyl and cyclohexyl; the R_1, R_2, R_3 and R_4 groups can contain heteroatoms such as O, S, and N. The inhibitors can be stereoisomers depending on the nature and spatial orientation of the groups surrounding X; two, three or four of the R_1, R_2, R_3 and R_4 groups can be linked together and have a bidentate, tridentate or tetradentate coordination with the central atom X; Alternatively, one, two, three or four of R_1, R_2, R_3 and R_4 can be Cl, Br, I, O, S, Se, carboxylate ions such as acetate and homologs, or other chemical ligands with an electron-donating group coordinated to X.
X= Ge, Sn, Pb or similar electrophilic atoms.

The GST inhibitors preferably contain tin (Sn) as electrophilic atom, since such compounds combine moderate toxicity with strong inhibition of the target enzyme. The tin atoms of the inhibitors can have different oxidation states, such as Sn(II) or Sn(IV), and the coordination number of the ligands can be 2, 3, 4, 5 or 6.

Preferably, one of R_1 –R_4 is Cl, Br or acetate and the other substituents are ethyl, butyl or phenyl.

A second group of inhibitors are steroids, steroid derivatives or steroid-mimetic compounds.

A third group of inhibitors are peptides, peptide derivatives or peptidomimetics with structural similarities to glutathione (γ-glutamyl-cysteinyl-glycine).

In a third aspect, the invention relates to a method for treating cancer or steroid hormone dependent diseases, comprising administering a compound that inhibits the enzymatic activity of GST A3-3/steroid isomerase (and/or GST A1-1) to a human in need of such a treatment. Such inhibition also includes reduction of the tissue level of active GST A3-3/steroid isomerase protein (and/or GST A1-1 protein). This reduction could be accomplished by inhibitory nucleic acid such as oligonucleotides, inhibitory RNA (siRNA or RNAi) or PNA (peptide nucleic acids) that have an effect on the gene expression and biosynthesis of the GST protein. Methods for suppression of gene expression by specific binding to the targeted gene or its corresponding RNA are well established within the field and reagents are commercially available for this purpose.

The human in need of the above-mentioned treatment may be an individual in need of treatment of steroid hormone dependent cancer or treatment of other steroid hormone dependent diseases, such as Cushing's syndrome.

In one embodiment the human is a male who suffers from prostate cancer. In another embodiment the human is a female who suffers from breast cancer.

Domestic animals (e.g. horse, dog) in need of steroid hormone suppression represent still another group of biological species to which the invention applies.

Brief description of the drawings

The invention will be described more closely below with reference to some non-limiting examples and figures.

Fig. 1. Metabolic pathways leading from cholesterol to steroid hormones such as testosterone (and further to dihydrotestosterone) and progesterone (and further to estradiol). The hormones act via binding to the androgen and estrogen receptors, respectively, and promote growth of hormone responsive prostate and breast cancer. GST A3-3 catalyzes essential steroid isomerizations in the respective pathways and the invention involves this enzyme as a target for hormone responsive disease.

Fig. 2. Alternative reactions for measuring the inhibition of GST A3-3 in vitro. All three reactions can be monitored spectrophotometrically using purified enzyme and glutathione (GSH): (A) Δ^5-androstene-3,17-dione; (B) 1-chloro-2,4-dinitrobenzene; and (C) phenethylisothiocyanate. Addition of an inhibitor will decrease the rate of the reaction catalyzed by GST A3-3.

Detailed description of the invention

Experimental procedures

Materials—1-Chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) can be purchased from Sigma (St. Louis, MO), phenethylisothiocyanate from Aldrich (Milwaukee, WI), and Δ^5-androstene-3,17-dione from Steraloids Inc. (Newport, RI).

Expression and purification of GSTs—Human GST A3-3 and its homologous GST proteins of the Alpha class were expressed from corresponding cDNA carried by the pET-21a(+) vector in *E. coli* BL-21(DE3) (2). The cells were grown to OD_{600}=0.7 and expression was induced by addition of 1 mM IPTG. The cells were grown for four hours, collected by centrifugation, and lysed using ultrasonication. The lysate was desalted on a PD-10 gel filtration column (Amersham Biosciences) and the proteins were eluted in 20 mM sodium phosphate, pH 7.0,

and were subsequently loaded onto a HiTrap SP cation exchanger (Amersham Biosciences). The proteins were eluted using a salt gradient. This single purification step yielded highly pure enzymes as confirmed by SDS-PAGE stained with Coomassie Brilliant Blue.

Specific activity measurements—The specific activities of GST A3-3 were determined for the isomerization reaction with Δ^5-AD (Fig. 2A), the conjugation reaction with 1-chloro-2,4-dinitrobenzene (CDNB) and GSH (Fig. 2B), and for the addition of GSH to phenethylisothiocyanate (Fig. 2C). The reactions were monitored spectrophotometrically at 30 °C. The isomerization of 100 μM Δ^5-AD was followed at 248 nm in 25 mM sodium phosphate buffer, pH 8.0, in the presence of 1 mM GSH. The extinction coefficient for the product Δ^4-AD is 16,300 $M^{-1}cm^{-1}$. Specific activity measurements were performed in 0.1 M sodium phosphate, pH 6.5, with 1 mM CDNB in the presence of 1 mM GSH as described (4), and with 0.1 mM phenethylisothiocyanate in the presence of 1 mM GSH (2).

Examples of specific inhibitors of Alpha class GSTs

Enzyme activities were determined in the standard assay system and the concentration of the inhibitor giving 50 % inhibition of the activity (IC_{50}) was determined.

Even if the compounds are inhibiting several GSTs, some inhibitors display high selectivity for a given GST (1). The present inventor has shown that this applies also to homologous members of the same GST class (Table 1). Selective inhibition is desirable to avoid interference with non-targeted GST-catalyzed reactions and to minimize possible toxic side effects. Without any extensive screening, inhibitors of GST A3-3 effective in the nanomolar concentration range have already been identified. These inhibitors also display selectivity among GST A3-3 and other human Alpha class members (Table 1). However, the related GST A1-1 has approximately 5% of the specific activity of GST A3-3 in the isomerization of androstenedione (2), and it may be advantageous to inhibit GST A1-1 in addition to GST A3-3. By use of multivariate cluster analysis of inhibition data it is possible to optimize discrimination among the enzymes.

Table 1. Differential inhibition of Alpha class glutathione transferases demonstrated by using organometallic compounds. The IC_{50} values are the inhibitor concentrations giving 50% inhibition of the GST-catalyzed reaction.

IC_{50} Values (μM)			
Inhibitor	GST A1-1	GST A2-2	GST A3-3
Et_3GeCl	56	0.8	67
Bu_3SnAc	0.018	0.41	0.018
Et_3SnBr	5.7	0.19	0.69
Ph_3PbCl	0.0046	0.084	0.013
Ph_3SnAc	0.16	Nd	0.16
Et_3PbCl	2	Nd	2.3
Ph_3PbBr	0.0086	Nd	0.16

Et, Bu, and Ph are ethyl, n-butyl, and phenyl, respectively; Nd = not determined.

Other examples of compounds inhibiting GST A3-3 is a steroid such as Δ^5-androsten-3β-ol-17-one or a structurally similar compound.

Other possible inhibitors that inhibit GST A3-3 can be found among peptides, peptide derivatives or peptidomimetic compounds having structural similarities with glutathione (i.e., γ-glutamyl-cysteinyl-glycine), and which are S-substituted, or otherwise substituted glutathione derivatives. Substituents include alkyl, aryl and aralkyl groups. Such inhibitors can for example be S-hexyl-glutathione or S-p-bromobenzyl-glutathione.

References

1. B. Mannervik and U.H. Danielson (1988) Glutathione transferases - structure and catalytic activity, CRC Crit. Rev. Biochem. 23, 283-337.

2. A.-S. Johansson and B. Mannervik (2001) Human glutathione transferase A3-3, a highly efficient catalyst of double-bond isomerization in the biosynthetic pathway of steroid hormones, J. Biol. Chem. 276, 33061-33065.

3. T . Stark, L. Mankowitz and J.W. DePierre (2002) Expression of glutathione transferase isoenzymes in the human H295R adrenal cell line and the effect of forskolin, J. Biochem. Mol. Toxicol. 16, 169-173.

4. B. Mannervik and P. Jemth (1999) Measurement of glutathione transferases, in "Current Protocols in Toxicology" (M.D. Maines, L.G. Costa, D.J. Reed, S. Sassa, and I.G. Sipes, eds.), pp. 6.4.1-6.4.10, John Wiley & Sons, New York.

CLAIMS

1. Method of screening for compounds that suppress the concentration of active glutathione transferase (GST) protein or inhibit the steroid isomerase activity of glutathione transferase (GST), wherein a glutathione transferase (GST) is used as a drug target.

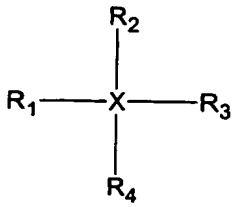
2. Method according to claim 1, wherein the GST used is GST A3-3.

3. Method according to claim 1, wherein the GST used is GST A1-1.

4. An inhibitor that inhibits the steroid isomerase activity of glutathione transferase (GST) identifiable by the method according to any of claims 1-3.

5. An inhibitor lowering the tissue concentration of active glutathione transferase (GST) identifiable by the method according to any of claims 1-3.

6. An inhibitor according to claim 4, wherein the inhibitor is a compound having the following formula:



wherein R_1, R_2, R_3 and R_4 can be alkyl groups, such as methyl, ethyl, propyl, butyl, pentyl, hexyl; aryl groups, such as phenyl or substituted phenyl, preferably substituted with lower alkyl, hydroxyl or alkoxy groups; or chemical derivatives or combinations of these groups; the R_1, R_2, R_3 and R_4 groups can be linear; branched, such as substituted with lower alkyl, hydroxyl or alkoxy groups; or cyclic, such as cyclopentyl and cyclohexyl; the R_1, R_2, R_3 and R_4 groups can contain heteroatoms such as O, S, and N; alternatively, one, two, three or four of R_1, R_2, R_3 and R_4 can be Cl, Br, I, O, S, Se, carboxylate ions such as acetate and homologs, or other chemical ligands with an electron-donating group coordinated to X;
X= Ge, Sn, Pb or similar electrophilic atoms;

as well as stereoisomers of the inhibitor.

7. An inhibitor according to claim 6, wherein X is Sn.

8. An inhibitor according to claim 6 or 7, wherein one of R_1- R_4 is Cl, Br or acetate and the other substituents are ethyl, butyl or phenyl.

9. An inhibitor according to claim 4, wherein the inhibitor is a steroid, steroid derivative or steroid-mimetic compound.

10. An inhibitor according to claim 9, wherein the inhibitor is Δ^5-androsten-3β-ol-17-one or a structurally similar compound.

11. An inhibitor according to claim 4, wherein the inhibitor is a peptide, peptide derivative or peptidomimetic compound with structural similarities to glutathione.

12. An inhibitor according to claim 11, wherein the inhibitor is an S-substituted, and/or otherwise substituted, glutathione derivative where the substituents may be alkyl, aryl and aralkyl groups.

13. An inhibitor according to claim 12, wherein the inhibitor is S-hexyl-glutathione or S-p-bromobenzyl-glutathione.

14. An inhibitor according to claim 5, wherein the inhibitor is an inhibitory nucleic acid such as an oligonucleotide, an inhibitory RNA (siRNA or RNAi) or PNA (a peptide nucleic acid).

15. An inhibitor according to claim 4-14 for use as a medicament.

16. A medicament according to claim 15 for use in treatment of steroid hormone dependent diseases in a mammal.

17. A medicament according to claim 16 for use in treatment of steroid hormone dependent cancer.

18. A medicament according to claim 17 for use in treatment of prostate cancer.

19. A medicament according to claim 17 for use in treatment of breast cancer.

20. A medicament according to claim 16 for use in treatment of Cushing's syndrome.

21. A method for treating cancer or steroid hormone dependent diseases, comprising administering a compound that suppresses the concentration of active glutathione transferase (GST) protein or inhibits the steroid isomerase activity of glutathione transferase (GST) of GST A3-3 and/or GST A1-1 to a human in need of such a treatment.

22. A method according to claim 21, wherein the human is a male who suffers from prostate cancer.

23. A method according to claim 21, wherein the human is a female who suffers from breast cancer.

24. A method according to claim 21, wherein the human is suffering from Cushing's syndrome.

FIG. 1

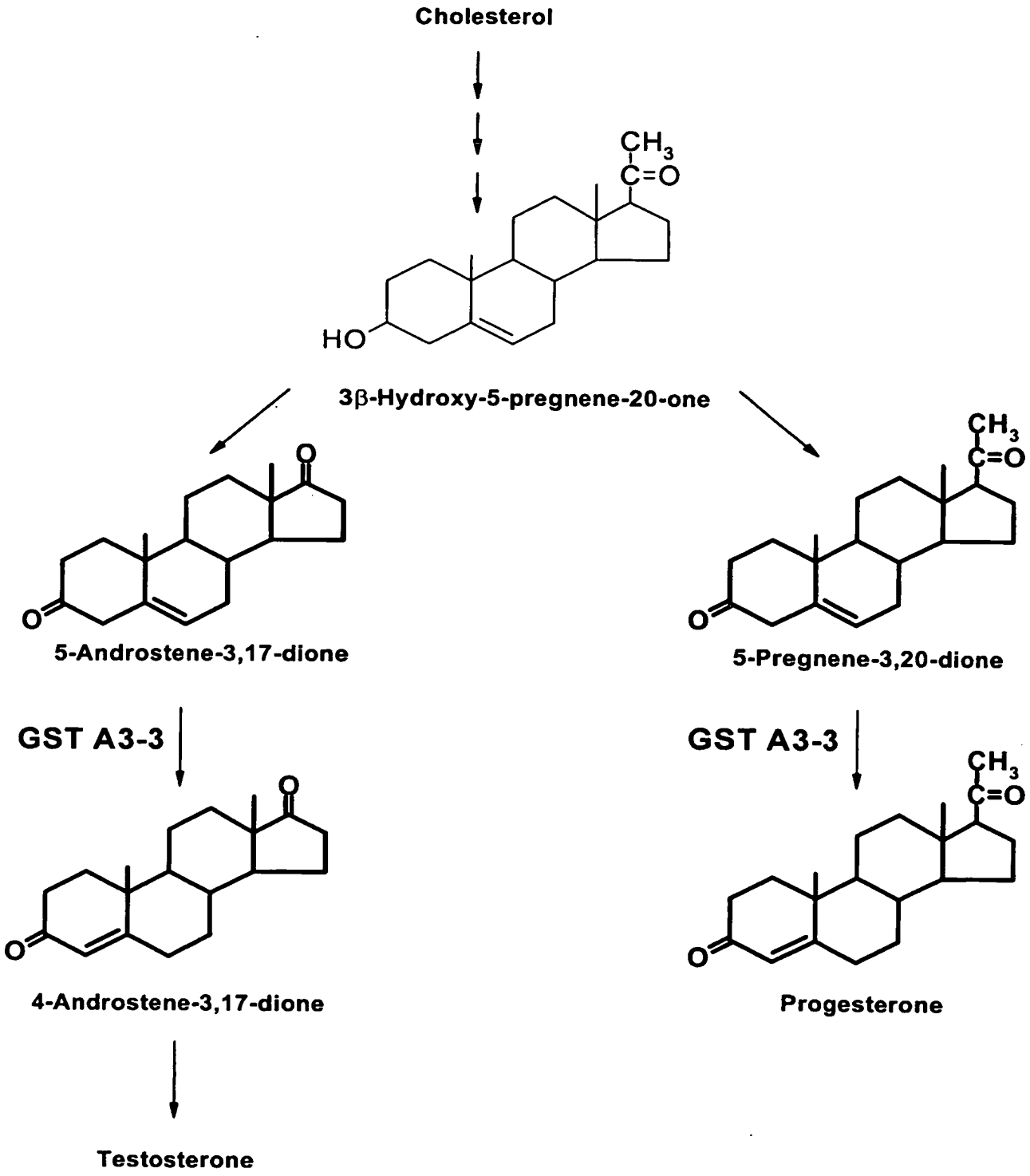
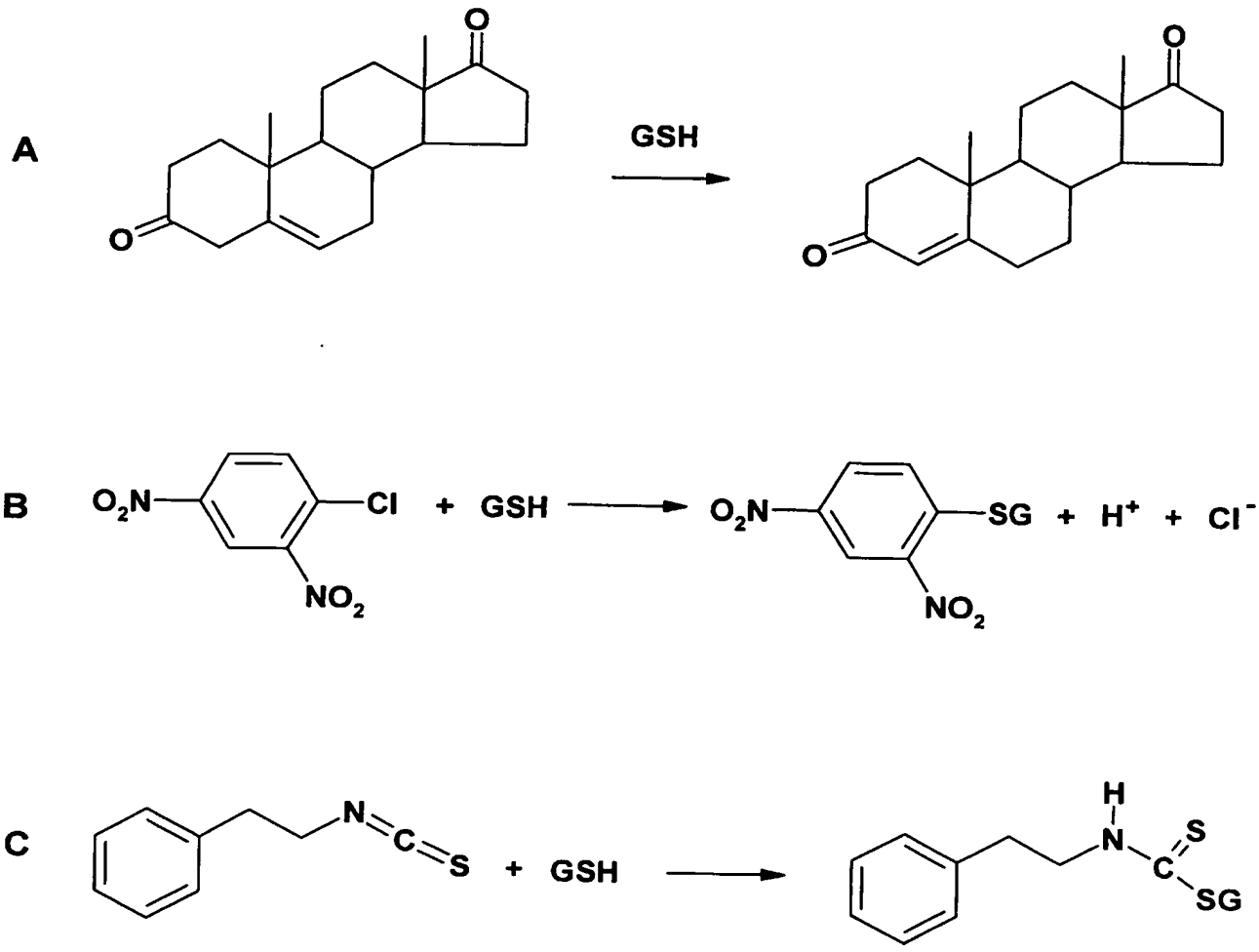

Cholesterol
CH3
C=O
HO
3β-Hydroxy-5-pregnene-20-one
O
O
5-Androstene-3,17-dione
CH3
C=O
O
5-Pregnene-3,20-dione
GST A3-3
O
O
4-Androstene-3,17-dione
GST A3-3
CH3
C=O
O
Progesterone
Testosterone

FIG. 2



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2003/001817

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/54, C12Q 1/48, C12N 9/10, C07K 14/47, C07D 209/14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N, C12Q, C07K, C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, EPODOC, WPI, MEDLINE, EMBASE, STN-CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biochemical Pharmacology, Volume 25, 1976, Roy A. Henry et al, "Inhibition of glutathione-S-aryltransferase from rat liver by organogermanium, lead and tin compounds", pages 2291-2295, page 2292, Results --	1-8,15-20
X	JBC Papers in Press. Published on February 28, 2002 as Manuscript M201062200, Copyright 2002 by the American Society of Biochemistry and Molecular Biology, Ann-Sofie Johansson and Bengt Mannervik, "Active-Site Residues Governing High Steroid Isomerase Activity in Human Glutathione Transferase A3-3",pages 1-35, Figure 1 --	1-8,15-20

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 February 2004	25 -03- 2004
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer FERNANDO FARIETA/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2003/001817

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 21-24
because they relate to subject matter not required to be searched by this Authority, namely:

see extra sheet

2. ☒ Claims Nos.: 11-14 and part of 1-6, 15-20
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see extra sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

7-8 and part of 1-6, 15

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Box II.1

Claims 21-24 relate to methods of treatment of the human or animal body by surgery or by therapy or diagnostic methods practised on the human or animal body (PCT Rule 39.1(iv)). Nevertheless, a search has been executed for those claims. The search has been based on the alleged effects of the compounds or medicaments.

Box II.2

Claims 1-5

Glutathione S-transferases (GSTs; EC 2.5.1.18) are ubiquitous multifunctional enzymes which play a key role in cellular detoxification. Based on their sequence homology, substrate specificity and immunological cross-reactivity, GSTs have been grouped into five species-independent classes of isoenzymes. Four of these classes (alpha, pi, mu and theta) comprise cytosolic enzymes, a fifth rather distinct form is microsomal. All cytosolic GSTs are found to be homo- or hetero- dimeric enzymes (from within the same class) with a relative molecular weight of ca. 50 kDa. (Krengel et al. (1998). FEBS Lett. 422, 285-290.)

Present claims 1-5 relate to a method of screening for compounds, wherein a glutathione transferase (GST) is used as a drug target. The method is only defined by reference to the following parameters:
P1: The compound suppress the concentration of active GST or,
P2: The compound inhibits the steroid isomerase activity of GST.

The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. Methods of screening in similar manners are described in Hiratsuka et al (Biochem J-2001-(355),237-234) for GST isoforms.
Methods of screening GST enzymes are known even in documents: WO 00/18937A1 and US6063570.

The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to:
The parts relating to the methods mentioned in the description on pages 5-7 (Table 1).

.../...

Claim 6

Present claims 6 relates to an extremely large number of possible compounds.
The expressions: "homologs" or "other chemical ligands" or "similar electrophilic atoms"; may influence the active compound in different ways. If such expressions influence the activity of the compound, it must be evident from the claim what groups are involved. In this case, claim 6 lacks definition of matter, which according to Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the variations claimed (See Table 1, description).

Claims 11-14 and part of 15-20

The information given in the working examples of the description is not considered to fulfil the requirements of exemplification in such a way that the claims are sufficiently supported (Articles 5 and 6 PCT Rules).

Consequently the search has been carried out for those parts of the claims, which appears to be supported, and disclosed, namely those parts related to the methods mentioned in the description on pages 5-7 (Table 1).

Box III

According to Article 17(2 and 3)and Rule 13.2 PCT, an international application shall relate to one invention only or to a group of inventions linked by one or more of the same or corresponding "special technical features", i. e. features that define a contribution which each of the inventions makes over the prior art. The present application relates to inhibitors of GST, according to the following two inventions, namely:

1- An inhibitor of GST with the formula I, wherein X=Sn according to claims 7-8 and part of 1-6,15.
2- An inhibitor of GST , wherein the inhibitor is a steroid according to claims 9-10 and part of 1-6,15-20.

Inhibitors of GST are known according to D1 (WO9632936A2). D1 relates to novel haloenol lactone compounds as inhibitors of GST. The compounds of D1 are useful for the specific measurement of particular isoenzymes of glutathione S-transferase and for the treatment of drug resistance in cancer. Thus, no special technical feature links inventions 1 and 2. The application is therefore not considered to fulfil the requirements of Rule 13.2 (Art 17.3) PCT.

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Invention 1 has been searched to the extend it was possible, based on the exemplified IC-50 measure of table 1 in the description.

INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	Biochem. J., Volume 360, 2001, David Sheehan et al, "Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily", pages 1-16, Conclusions	1-8,15-20
Y	Biochem. J., Volume 355, 2001, Akira Hiratsuka et al, "(S)-Preferential detoxification of 4-hydroxy-2(E)-nonenal enantiomers by hepatic glutathione S-transferase isoforms in guinea-pigs and rats", pages 237-244, Tables 1-3	1-8,15-20
A	WO 9520601 A1 (THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL), 3 August 1995 (03.08.1995), page 29 - line 34	1-8,15-20
A	WO 0018937 A1 (E.I DU PONT DE NEMOURS AND COMPANY), 6 April 2000 (06.04.2000), claim 13	1-8,15-20
A	US 6063570 A (MCGONIGLE ET AL), 16 May 2000 (16.05.2000), claims 7,8,9	1-8,15-20
A	FEBS Letter, Volume 422, 1998, Krengel et al, "Glutathion S-transferase (GSTA4-4), pages 285-290, abstract http://www.csb.gu.se/ute/projects/gst.html	1-8,15-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2003/001817

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9632936 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 24 October 1996 (24.10.1996), claim 29	1-8,15-20

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